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THE EFFECT OF THERMAL PROCESSING ON ANTIOXIDANT ACTIVITY AND TOTAL POLYPHENOLS IN JERUSALEM ARTICHOKE (*Helianthus tuberosus* L.)

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Abstract

The Jerusalem artichoke (Helianthus tuberosus L.) is high in protein, inulin, and other bioactive ingredients. In this study, the effect of heat treatment methods as well as the effect of the variety on its total polyphenol content and antioxidant activity were investigated. The total polyphenol content ranged from 0.36 to 0.87 mg GAE.g^{-1} DW in raw flesh and from 0.41 (baking) to 1.01 mg GAE.g^{-1} DW (boiling) in heat-treated tubers. The antioxidant activity (AA) was determined by the methods of DPPH and FRAP. DPPH values ranged from 0.18 to 1.09 (raw flesh), 3.03 to 4.74 (boiled samples), 2.34 to 2.94 (baked samples), 2.88 to 3.22 (microwaved samples), and 2.25 to 6.17 (steamed samples) µmol TE.g^{-1} DW. AA values by the method FRAP ranged from 3.5 to 3.82 (raw flesh), 7.51 to 8.53 (boiled samples), 4.26 to 6.80 (baked samples), 4.98 to 6.72 (microwaved samples), and 4.89 to 8.04 (steamed samples) µmol TE.g^{-1} DW. All studied heat treatment methods had a positive effect on the TPC and AA. Our results confirm the promising potential of artichoke use in functional food preparation.

Key words*: Jerusalem artichoke, heat treatment, polyphenols, antioxidant activity.*

INTRODUCTION

Originally from North America, the Jerusalem artichoke is an invasive perennial weed that has been brought as a cultivated plant to Europe and Asia (Liava et al., 2021). The *Asteraceae* family and *Helianthus* genus include the Jerusalem artichoke (*Helianthus tuberosus* L.), which has a high level of pest and disease resistance. It can withstand freezing temperatures and drafts. This plant has been grown for many years as a valuable edible raw material with potent medicinal properties (Sawicka et al., 2020).

The tubers can be oval in shape, but they can also be rounded, oblong, pear-shaped, or thin. They can be white, red, light brown, brown, violet-brown, or dark brown. Numerous cultivation parameters, such as plant variety, available moisture, and soil texture, influence these variations. While some varieties produce small tubers on long stems, relatively large tubers are grouped close to the main root in the better varieties (Kosaric et al., 1984; Liava et al., 2021).

The tubers of Jerusalem artichokes are an excellent source of phytochemicals. Jerusalem artichokes are highly suited for the obtaining of functional foods due to their nutritional composition (Michalska-Ciechanowska et al., 2019). Tubers store 6-12% protein, amino acids, and inulin-type fructans, which are polymers of fructose molecules (Dias et al., 2016).

The most prevalent carbohydrate in Jerusalem artichoke tubers and stems is inulin. The degree of polymerization, or number of units, of this compound typically ranges from 2 to 60, and the amounts of it in tubers vary amongst genotypes (Liava et al., 2021).

The Jerusalem artichoke tuber has an inulin content ranging from 7 to 30% of its fresh weight or 60 to 85.5% of its dry weight (Samal et al., 2017). Because Jerusalem artichokes have a high inulin content, they can be used to make diabetic foods such as flour, juice, syrup, confections, and bakery goods. Dietary fibre and inulin can absorb a large quantity of glucose from food and stop it from entering the bloodstream, lowering blood sugar levels.

When Jerusalem artichokes are used, cholesterol levels are significantly lowered. Inulin stimulates the body's bifidobacteria to grow rapidly, inhibits the growth of harmful

microflora, and aids in the restoration of the gastrointestinal tract's disrupted function (Shariati et al., 2021). Jerusalem artichokes are an excellent supplier of essential minerals in sufficient quantities for human nutrition. Greater than other tubers (carrots, potatoes, turnips, beets, etc.) in terms of iron content is the Jerusalem artichoke. Jerusalem artichokes also include minerals like silicon, magnesium, calcium, potassium, sodium, fluorine, and chromium (Ishniyazova et al., 2020; Rushchitc et al., 2022).

Jerusalem artichokes are a multipurpose crop that can be used for biomass and bioenergy production (bioethanol and biogas), pharmaceutical applications, and human food consumption (directly as tubers or as a raw material for obtaining sweeteners). Furthermore, Jerusalem artichokes have potential as a crop for fodder. Tubers are used for functional food component preparation because they are high in protein, inulin, and other bioactive ingredients (Kosaric et al., 1984; Dias et al., 2016; Mahokhina et al., 2022).

Growing times vary from 110 to 240 days, depending on the region and genotype under cultivation. Harvesting should be done after stem drying if Jerusalem artichoke is grown for tuber production, while if the aerial part is the primary product, harvesting can take place while tuber bulking is underway. In order to produce bioethanol, the stems in middle-season/late clones should be harvested at the flower bud stage, and in early clones, between the flower bud and dry head stages. This is because the stem's sugar content decreases beyond these stages (Liava et al., 2021).

The primary elements influencing the bioavailability of polyphenols are the food matrix, food processing, and the initial content of polyphenols in foods. Most vegetables and fruits are eaten in processed form. Boiling, baking, steaming, and other forms of industrial or domestic heat processing affect the amount of polyphenols present as well as their bioaccessibility and bioavailability (Arfaoui, 2021).

This study analyzed how the Jerusalem artichoke variety's overall polyphenol content and antioxidant activity were affected by various heat treatment techniques, including baking, boiling, steaming, and microwave cooking.

Figure 1: Jerusalem artichoke – plant (https://as2.ftcdn.net/v2/jpg/03/53/28/33/1000_F_353283302_Maarw5jeQKpEsBg3whLkcPBbVcZcsaE4.jpg)

MATERIALS AND METHODS

Chemicals

The following materials were acquired from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Steiheim, Germany): methanol (99.8%), gallic acid (p.a.), DPPH (2,2´-diphenyl-1-picrylhydrazyl), Trolox (2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), HCl, and acetic acid. Na_2CO_3 and $FeCl_3$ were purchased from CentralChem (Slovakia); sodium acetate was supplied by Mikrochem (Slovakia); and Folin-Ciocalteu reagent was acquired from Merck (Merck KGaA, Darmstadt, Germany).

Plant Material

For analysis, three Jerusalem artichoke varieties (pink, white 1, and white 2) grown in the same location (Slovakia), one of them with a different peel colour, harvested in September 2021, were used. In total, two kilograms of fresh Jerusalem artichokes from each variety were utilized for the heat treatment and extract preparation process.

Samples preparation

After a thorough cleaning, distilled water (dH_2O) was used to wash the Jerusalem artichokes.

After that, the tubers underwent peeling. Peeled Jerusalem artichokes (flesh) were cut into slices that were roughly 3 mm thick after being repeatedly cleaned in dH_2O. A portion of the raw flesh from the tubers was homogenised and mixed using a Grindomix GM 200, Retsch, Haan, Germany, for 30 seconds. The remaining portions of the tuber underwent the following heat-treatment procedures, as described by Musilová et al. (2020): steaming (15 min, 97 ± 2 °C), boiling (10 min), baking (15 min, 200 °C), and microwaving (5 min, 800 W). Following each heat treatment, Jerusalem artichoke slices were homogenised, cooled, and mixed (using a Grindomix GM 200, Retsch, Haan, Germany; 30 seconds). The homogenised samples were prepared into extracts needed for analysis using 80% methanol.

50 mL of 80% methanol was added to 25 g of homogenised material (raw, microwaved, steamed, boiled, and baked flesh, respectively) in order to prepare the extracts. The samples were extracted using a horizontal shaker (Heidolph Promax 1020, Heidolph Instruments GmbH, Schwabach, Germany) for a duration of 12 hours. After being filtered through Muktell No. 392 paper (Munktell & Filtrac GmbH, Bärenstein, Germany), the extracts were refrigerated at 4 °C in closed 50 mL centrifuge tubes.

Analyses of samples

Determination of total polyphenol content

Using spectrophotometry (UV-VIS spectrophotometer T92+, PG Instruments, Leicestershire, United Kingdom), the total polyphenol content (TPC) was ascertained using a Folin-Ciocalteu agent according to the method by Lachman et al. (2006). To the volume of sample extract (0.1 mL) in the volumetric flask (50 mL), the Folin-Ciocalteu reagent was added. After three minutes, 5 mL of 20% sodium carbonate aqueous was added, and distilled water was added to the mark. Standard gallic acid solutions were prepared for the calibration curve using the same procedure. After mixing the prepared solutions, they were kept at room temperature for two hours. Following that, a measurement of the solutions' absorbance at 765 nm was made. Each sample's polyphenol content was reported as milligrammes of gallic acid equivalent per gramme of dry weight (mg GAE.g^{-1} DW).

Evaluation of Antioxidant Activity

The DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) assay were used to measure the antioxidant activity (AA).

DPPH Radical Scavenging Activity

According to Brand-Williams et al. (1995), the method for determining antioxidant activity (AA) was based on scavenging the stable free radical 2,2´-diphenyl-1-picrylhydrazyl (DPPH). A 100 mL flask containing 0.025 g of DPPH free radical was filled with 99.8% methanol to create a stock solution, which was then kept in the cold and dark. The stock solution for DPPH was combined with methanol (1:10) to create the DPPH working solution needed for analysis. At a wavelength of 515.6 nm, the absorbance (A0) of the DPPH working solution was measured using a UV-VIS spectrophotometer T92+ (PG Instruments, Leicestershire, United Kingdom) for the analysis. After carefully mixing 0.1 mL of the sample extract into the DPPH solution in the cuvette, it was left to stand in the dark for 10 minutes. Subsequently, the absorbance (A10) was determined. The absorbance of the DPPH solution (A0) and the absorbance after adding the sample extract at time t = 10 minutes (A10) were used to calculate the percentage values of DPPH inhibition for each sample using the following formula:

$$\% \text{ DPPH inhibition} = [(A0 - A10) / A0] \times 100$$

Trolox equivalents (μmol TE.g^{-1} DW) are units of measurement for the antioxidant activity assessed using the DPPH method.

Ferric reducing antioxidant power assay

To make the FRAP reagent, a 1:1:10 ratio of TPTZ solution (5 mmol.L^{-1} in 40 mmol.L^{-1} HCl), ferric chloride solution (10 mmol.L^{-1}), and acetate buffer (acetic acid, c = 0.1 mol.L^{-1}; sodium acetate, c = 0.1 mol.L^{-1}, pH 3.6) was used. The following procedure was used to prepare the sample solutions for determination. Test tubes were filled with 6 mL of FRAP reagent and 0.1 mL of each sample extract, then they were homogenized and closed. After preparing the samples in this way, they were placed in a dark, 37 °C water bath for 30 minutes. Using the same process as the samples, standard solutions of Trolox were created for the calibration curve. An UV-VIS spectrophotometer T92+ (PG Instruments, Leicestershire,

United Kingdom) was then used to measure the absorbance at 593 nm.

Trolox equivalents per gram of dry weight (μmol $TE.g^{-1}$ DW), was the unit of measurement for the ferric reducing antioxidant power.

Statistical evaluation

There were four replicates of each analysis (n = 4). The arithmetic mean ± standard deviation (SD) is used to express the results. Every variable under test had a nonparametric distribution. Thus, to ascertain the statistical differences ($p < 0.05$) between varieties and heat treatment techniques, the Kruskal-Wallis test was employed. To ascertain the relationship between the three parameters under investigation (TPC, DPPH, and FRAP), Spearman's correlation coefficient was employed. The XLSTAT 2014 software package was used to carry out the computational work.

RESULTS AND DISCUSSIONS

Total polyphenol content

One significant class of secondary plant metabolites with a variety of functions in plants is represented by polyphenols. In general, genetics determines the amount and presence of polyphenols (Franková et al., 2022b). The cultivar, harvest time, and storage conditions all affect the amount of polyphenols in Jerusalem artichoke tubers. Genetics also influences variations in polyphenolic compound concentrations (Showkat et al., 2019). The raw Jerusalem artichoke flesh samples under investigation had total polyphenol content (TPC) ranging from 0.359 (White 1) to 0.868 mg $GAE.g^{-1}$ DW (Pink). TPC in the pink-fleshed variety (Pink) was two times higher than in white-fleshed (White 1 and White 2) Jerusalem artichokes (Table 1). Varieties with coloured flesh have higher total polyphenol contents because compared to varieties with white or yellow flesh, they have higher proportions of anthocyanins (Franková et al., 2022a).

Comparisons with previous studies revealed differences in the measured TPC values. A higher total polyphenol content was determined in the Kaentawan #1 variety from Thailand (2.81 mg $GAE.g^{-1}$ DW) (Puyanda et al., 2020). Additionally, Amarowicz et al. (2020) in their study examined, like us, two varieties of white and one variety of pink Jerusalem artichoke tubers. The authors found that the TPC content in the tubers was 7.81–9.76 $mg.g^{-1}$. The differences in our results may have been influenced by the different preparations of the extract because the authors prepared the extract from freeze-dried samples. In the Norwegian variety Dagnøytral, the content of polyphenols was in the range of 1.1–2.3 mg $GAE.g^{-1}$ DW (Showkat et al., 2019). In the water extract of Jerusalem artichoke, variety Patate, grown in Algeria, the TPC content was 6.58 mg $GAE.g^{-1}$ DW (Sarsar et al., 2021). In an additional research (Nizioł-Łukaszewska et al., 2018), the authors investigated the content of TPC in tubers and leaves of Jerusalem artichoke, and the results revealed a greater amount of TPC in the leaves of the plant (389.88 mg $GAE.g^{-1}$) than in the tubers (76.84 mg $GAE.g^{-1}$). The use of ultrasound-assisted extraction methods could have influenced the results of TPC content compared to our and other studies. Values in accordance with our results were reported by the study of Plangklang & Tangwongchai (2011), which also reported low TPC values (between 0.327 and 0.510 mg $GAE.g^{-1}$ FW) in varieties HEL65 and JA89. Our TPC values in fresh matter ranged from 0.359 to 0.868 mg $GAE.g^{-1}$. TPC increased in varieties with white skin (White 1, White 2) as a result of the carefully supervised heat treatments (steaming, boiling, baking, and microwaving). In the case of tubers with pink skin (Pink), the TPC increased only after the heat treatment of boiling, while the other thermal treatments led to a decrease of TPC in this sample. Sarsar et al. (2021) cooked tubers at 70°C for 10 min, and TPC values increased up to twofold to 13.11 mg $GAE.g^{-1}$ DW, as in the case of our white varieties (White 1, White 2). Other authors (Puyanda et al., 2020) baked tubers in an oven at 65 °C, and the TPC values increased (from 2.81 to 4.37 mg $GAE.g^{-1}$ DW), similar to our case. After microwaving in the research of Showkat et al. (2019), the TPC content increased slightly (1.5–3.5 $GAE.g^{-1}$ DW), and upon boiling (60 °C), TPC also increased (1.7–3.8 $GAE.g^{-1}$ DW). Microwaving and steaming are examples of heat treatments that can disrupt cell structure and increase the efficiency of compound extraction from the cell cytoplasm (Franková et al., 2022b). Complex

carbohydrates and proteins contain phenolic compounds. The species, variety, agrotechnical, climatic, and, in particular, plant parts can all affect the composition of the phenolic fraction in natural plant sources (Nizioł-Łukaszewska et al., 2018).

In Table 1, the relationships between TPC and heat treatments are indicated by different letters (a, b). Statistically significant differences were observed between raw and boiled samples (p = 0.0048), as well as between heat treatment by boiling and baking (p = 0.0015).

Table 1. TPC and AA in raw and heat-treated Jerusalem artichoke

Variety	Heat treatment	TPC (mg GAE.g^{-1} DW)	Antioxidant activity		
			DPPH (µmol TE.g^{-1} DW)	DPPH (%)	FRAP (µmol TE.g^{-1} DW)
Pink	Raw flesh	0.868 ± 0.114^{a}	1.085 ± 0.108^{a}	13.99	3.504 ± 0.536^{a}
White 1		0.359 ± 0.113^{a}	0.183 ± 0.055^{a}	6.58	3.687 ± 0.360^{a}
White 2		0.487 ± 0.149^{a}	0.302 ± 0.297^{a}	8.74	3.821 ± 0.163^{a}
Pink	Steaming	0.816 ± 0.228^{ab}	6.168 ± 0.106^{b}	69.17	8.022 ± 0.376^{bc}
White 1		0.566 ± 0.112^{ab}	2.253 ± 0.187^{b}	31.43	4.888 ± 0.119^{bc}
White 2		0.922 ± 0.084^{ab}	5.267 ± 0.160^{b}	62.08	8.040 ± 0.535^{bc}
Pink	Boiling	1.009 ± 0.158^{b}	4.745 ± 0.300^{b}	69.53	8.533 ± 0.411^{bc}
White 1		0.860 ± 0.192^{b}	4.013 ± 0.198^{b}	45.69	8.014 ± 0.716^{bc}
White 2		0.863 ± 0.445^{b}	3.027 ± 0.183^{b}	17.02	7.507 ± 0.233^{bc}
Pink	Baking	0.515 ± 0.143^{a}	2.942 ± 0.117^{ab}	76.88	4.744 ± 0.107^{ab}
White 1		0.406 ± 0.046^{a}	2.341 ± 0.208^{ab}	55.90	4.260 ± 0.059^{ab}
White 2		0.738 ± 0.194^{a}	2.737 ± 0.078^{ab}	79.62	6.803 ± 0.719^{ab}
Pink	Microwaving	0.644 ± 0.092^{ab}	2.877 ± 0.042^{b}	75.49	4.983 ± 0.271^{b}
White 1		0.484 ± 0.135^{ab}	3.095 ± 0.208^{b}	66.24	5.162 ± 0.468^{b}
White 2		0.689 ± 0.047^{ab}	3.219 ± 0.070^{b}	74.95	6.724 ± 0.133^{b}

Legend: TPC – Total polyphenol content, GAE – gallic acid equivalent, DW – dry weight, DPPH – 2,2′-diphenyl-1-picrylhydrazyl, TE – Trolox equivalents, FRAP – Ferric reducing antioxidant power. The values are expressed as average ± SD. Different letters (a-c) indicate significant differences among heat treatments, for each method separately.

Antioxidant activity

An analysis of the antioxidant activity of natural products serves as the foundation for the evaluation and recommendation of foods with high antioxidant activity to consumers (Xu et al., 2017). The ferric reducing antioxidant power (FRAP) assay and DPPH free radical scavenging activity were used to assess the antioxidant activity (AA) of Jerusalem artichoke tubers. As individual bioactive compounds have a different affinity for different antioxidant activity tests, the sensitivity and specificity of one method of antioxidant activity determination do not provide a complete representation of all the antioxidants in the sample (Makori et al., 2020).

AA values of DPPH varied between 0.18 and 1.09 (raw flesh), 3.03 to 4.74 (boiled samples), 2.34 to 2.94 (baked samples), 2.88 to 3.22 (microwaved samples), and 2.25 to 6.17 (steamed samples) µmol TE.g^{-1} DW. The AA values obtained using the FRAP method varied from 3.5 to 3.82 (raw flesh), 7.51 to 8.53 (boiled samples), 4.26 to 6.80 (baked samples), 4.98 to 6.72 (microwaved samples), and 4.89 to 8.04 (steamed samples) µmol TE.g^{-1} DW (Table 1). The steaming heat treatment affected the antioxidant activity in the samples most significantly compared to the raw flesh. Jerusalem artichokes' total polyphenol content and antioxidant activity were positively impacted by all of the heat treatment techniques that were investigated.

The antioxidant activity investigated in a study in Poland (Amarowicz et al., 2020) showed that AA values measured by the DPPH method were in the range of 40.9–46.2 µmol.g^{-1}. Nizioł-Łukaszewska et al. (2018) reported that Jerusalem artichoke tubers had a free radical scavenging capacity of 56%. Boiled tubers at a temperature of 60°C and also raw flesh in the work of Sarsar et al. (2021) showed AA in the range of 83–99.54%. In another study, the authors (Mu et al., 2021) observed the effect of different tuber storage temperatures on AA. The tubers were kept in storage between -18 and

4°C, while the percentage of free radical absorption ranged from 39.08 to 84.04%. Raw flesh and heat-treated samples of our variants had this ability in the range of 6.58%–79.62%, so the authors report similar AA results. Other authors (Alyas et al., 2021; Puyanda et al., 2020; Plangklang & Tangwongchai, 2011; Zhang & Kim, 2015) also examined the antioxidant activity using the FRAP and DPPH techniques; however, due to the difference in the reported units, it is not possible to compare these results. According to some theories, oxidation, leaching, thermal degradation, and enzymatic or nonenzymatic conversion can all contribute to the loss of antioxidant activity that happens when fruits and vegetables are processed. The softening of the food matrix during cooking, on the other hand, has been linked to increased antioxidant activity during processing. This increases the extractability of the antioxidant constituents, leading them to further convert to more antioxidant compounds (Amagloh et al., 2022).

The Kruskal-Wallis test, a statistical analysis, confirmed that there were differences ($p < 0.05$) in all examined antioxidant activity parameters (DPPH and FRAP) between heat-treated and raw flesh. Statistically significant differences in AA by DPPH were observed between raw flesh and steaming, boiling, and microwaving, respectively. The same statistically significant differences were also found with the FRAP method, but significant differences were also found between the heat treatments of boiling and baking and boiling and microwaving.

The Spearman's correlation coefficient

Relationships between the two monitored parameters - total polyphenol content and antioxidant activity (DPPH and FRAP) - were ascertained using Spearman's correlation coefficient. According to our findings, positive correlations were found for every parameter that was examined. TPC showed a positive correlation ($r = 0.57$) with both FRAP ($r = 0.56$) and DPPH free radical scavenging activity. The two antioxidant activity methods, DPPH and FRAP, showed a very strong positive correlation ($r = 0.84$, $p < 0.0001$). Thus, it can be inferred that the predictive power of both techniques for Jerusalem artichoke's antioxidant activity is similar.

CONCLUSIONS

Our results confirm the promising potential of Jerusalem artichoke use in functional food preparation. All of the processing techniques that were investigated had a positive effect on the overall polyphenol content and antioxidant activity. The variations between the heat treatment techniques and the varieties under observation were validated through statistical analysis. Our study's data can contribute to a deeper understanding of how processing affects the bioactive compound content and antioxidant activity of Jerusalem artichokes.

This plant could be usefull for the development and production of nutritious and extremely successful therapeutic and preventive foods. A good way to expand the range of alternative uses for root vegetables is to incorporate them into different food products.

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